Atty. Dkt. No. 017348/0361

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Keiji ENPUKU

Title: A METHOD FOR AN IMMUNOASSAY WITH A MAGNETIC LABEL AND AN APPARATUS FOR THE SAME

Appl. No.: Unassigned

Filing Date: 07/21/2000

Examiner: Unassigned

Art Unit: Unassigned

UTILITY PATENT APPLICATION TRANSMITTAL

Assistant Commissioner for Patents
Box PATENT APPLICATION
Washington, D.C. 20231

Sir:

Transmitted herewith for filing under 37 C.F.R. § 1.53(b) is the nonprovisional utility patent application of:

Keiji ENPUKU

Enclosed are:

[**X**] Specification, Claim(s), and Abstract (13 pages).

[**X**] Informal drawings (6 sheets, Figures 1-7).

[**X**] Assignment of the invention to SUMITOMO ELECTRIC INDUSTRIES, LTD. And MTI CO., LTD.

[**X**] Assignment Recordation Cover Sheet. (2 pgs.)

[**X**] Declaration and Power of Attorney (2 pages).

[**X**] Preliminary Amendment (2 pgs.)

The filing fee is calculated below:

	Claims as Filed		Included in Basic Fee		Extra Claims		Rate		Fee Totals
Basic Fee							$690.00		$690.00
Total Claims:	11	-	20	=	0	x	$18.00	=	$0.00
Independents:	2	-	3	=	0	x	$78.00	=	$0.00
If any Multiple Dependent Claim(s) present:						+	$260.00	=	$0.00
							SUBTOTAL:	=	$690.00
[ ]	Small Entity Fees Apply (subtract ½ of above):							=	$0.00
	Assignment Recordation fee:							=	$40.00
	TOTAL FILING FEE:							=	$730.00

[**X**] A check in the amount of $730.00 to cover the filing fee is enclosed.

[ ] The required filing fees are not enclosed but will be submitted in response to the Notice to File Missing Parts of Application.

[**X**] The Assistant Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Assistant Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741.

Please direct all correspondence to the undersigned attorney or agent at the address indicated below.

Respectfully submitted,

Date July 21, 2000

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PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified application, Applicant respectfully requests that the following amendment be entered into the application:

In the Specification:

Page 5, line 12, delete "Figure 3 shows" and substitute therefor --Figures 3(a) and 3(b) show--.

REMARKS

Entry of the foregoing amendment prior to examination is respectfully requested.

Respectfully submitted,

Date July 21, 2000

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Specification

Title of the Invention

A method for an immunoassay with a magnetic label and an apparatus for the same

Detailed description of the Invention

Field of the Invention

The present invention relates to an immunoassay and an apparatus for the same. More specifically, the present invention relates to a method and an apparatus for an immunoassay with a magnetic label and a SQUID.

Detailed description of invention

An immunoassay is a method to detect an antigen or an antibody (mentioned with word "analyte" in this specification). For identification or measurement, a label is attached to antibody of antigen antibody reaction. Various labels and detection method are executed frequently and proposed.

Particularly, various optical methods are known well. In these methods, labels with light, fluorescence or color are used. However, optical methods have short sensitivity for requirement.

As an another method, method with radioactive label is known. However, this method is pointed out a problem about safety and limited its execution.

Furthermore, there is methods with magnetic labels as a reemergence measurement or a magnetic relaxation method. However, in this method, grain size of the label influences to measured value

seriously. Therefore, accuracy of measurement of this method should not be stable.

On the other hand, a SQUID was put to practical use recently. The SQUID comprises a circular current road and one or two Josephson junction(s) on the road. The SQUID has a very high sensitivity compared with a Hall device or a flux gate and is used as a magnetism sensor.

Then, a new assessment method with magnetic label occurs to us. In this method, it is expected that labels are detected by a SQUID with high accuracy. However, there is no practical method with a SQUID. Magnetic label has to be magnetized for detection by a SQUID. However, a strong magnetic field of dozens of gauss dimension is necessary for label to be magnetized.

On the other hand, a SQUID has very high sensitivity. Therefore, a serious problem occurs that a SQUID receives affect of magnetic field of magnetization means and measured value changes.

Furthermore, an analyte is treated with prepared slide actually. But, a strong magnetic field magnetizes a prepared slide. Therefore, it is difficult to detect only a label.

Summary of the Invention

Then the present invention provides a method for an immunoassay with magnetized label and SQUID, which comprising following processes;

(1) an analyte is labeled with a magnetic material label to detect antigen-antibody reaction,

(2) the magnetic material label is magnetized by a magnetic field,

(3) the magnetized magnetic material label detected by a SQUID which detect a magnetic field having right angle to the magnetic

field.

In method of the present invention, labels are magnetized and detected by a SQUID. According to a preferable embodiment of the present invention, the magnetic field for magnetization is a static magnetic field.

According to another preferable embodiment of the present invention, an analyte is inspected while moving parallel to the flux forming the magnetic field inside the detection region of the SQUID. Then, the SQUID detects a variation of magnetic field occurred by the moving labels magnetized in particular direction.

At the same time, the present invention contains an apparatus to execute the method provided by the present invention. The apparatus comprises a magnetic field generation means that generates a magnetic field to magnetize the labels. The apparatus comprises a SQUID that measures magnetic field.

It is preferable that the apparatus comprises a transportation means which moves the analyte with magnetized label parallel to the magnetic field generated by the magnetic field generation means.

Furthermore, the apparatus comprises magnetic field compensation means preferably The compensation means generates a magnetic field parallel to the detection direction of the SQUID. The magnetic field for compensation cancels the magnetic field that has right angle to the magnetic field for magnetization. Because, the magnetic field for magnetization contains component that has right angle to the desired magnetic field and the SQUID has very high sensitivity to detect the component.

According to the preferable embodiment of the present invention,

the SQUID is formed of an oxide superconducting thin film having a high critical temperature. By the way, the sensitivity of a SQUID is in proportion to 3 power of distance between a SQUID and an analyte. The oxide superconducting materials can be used with a small cooling-systems. The use of the oxide superconducting materials is advantageous in this point.

It is an important characteristic of the present invention that the magnetic field for magnetization has right angle to the magnetic field detected by the SQUID. That is to say, in a prior art, the magnetic field for magnetization and the magnetic field detected are parallel each. Therefore, the SQUID detects a magnetic field for magnetization, too.

On the contrary, in apparatus of the present invention, the magnetic fields are arranged right angle each. The SQUID detects a flux having right angle to its circular current road and never detects a flux parallel to the circular current road. Therefore, in apparatus provided by the present invention, the SQUID does not detect the magnetic field for magnetization. In a method with a SQUID by prior art, a magnetic field for magnetization is alternative and a noise is offset by using a lock in amplifier.

According to a preferable embodiment of the present invention, a static magnetic field can be used. Because, the static magnetic field can be easily compensated by simple means with a solenoid.

However, because the SQUID has very high sensitivity, even using the magnetic field for compensation will not compensate the magnetic field for magnetization perfectly. Then, according to a preferable embodiment of the present invention, the SQUID detects a variation of magnetic field. This variation of magnetic field is occurred by a motion

of the magnetized label in the detection field. This variation itself is not influenced magnetic field of perimeter.

The above and other objects, features and advantages of the present invention will be apparent from following description of preferred embodiments of the invention with reference to the accompanying drawings.

Brief Description of the Drawings

Figure 1 is a perspective view showing a principle of the method provided by the present invention.

Figure 2 is a sectional view showing a basic construction of the apparatus provided by the present invention.

Figure 3 shows labels and antibodies.

Figure 4 is a graph showing an output signal of the SQUID.

Figure 5 is a graph showing a relationship between concentration of an antibody and the output of the SQUID.

Figure 6 shows the antigen-antibody reaction labeled with a magnetic label.

Figure 7 is a graph showing measured resultant in comparison with a resultant by a prior art.

Description of the Preferred embodiments

In the method of the present invention, as shown in the figure 1, an analyte 2 supported on a support 1 with label is magnetized at first by a magnetic field shown with arrow A parallel to surface of the support 1. and is detected by a SQUID 3 at last.

The SQUID 3 comprises a ringed current road that is arranged parallel to the surface of the support 1. Therefore, a magnetic flux

detected by the SQUID 3 has a right angle to the surface of the support 1. Namely, a region under the SQUID 3 becomes a detection region of the SQUID 3. On the contrary, the magnetic field for magnetization is parallel to the surface of the support 1. Therefore, the SQUID 3 has no sensitivity to the magnetic field A for magnetization substantially.

Furthermore, the support 1 moves parallel to the magnetic field A with fixed velocity X. When the analyte 2 passes into the detection region of the SQUID 3, the magnetic field of the detection region changes and the SQUID 3 detects the change of the magnetic field. By the way, at the same time, the support 1 is magnetized too. Therefore, it is preferable that the length L and the width W of support 1 are large sufficiently so that the detection region is met by support 1 while no analyte 2 is in the detection region.

The method mentioned above can be executed with an apparatus shown by figure 2. This apparatus comprises magnetic shields 101 a, 101 b, SQUID 103, coils for magnetization 106 a, 106 b, a compensating coil 107 and a transportation means 105.

The magnetic shields 101a, 101b surround the whole apparatus and the measurement is done within the magnetic shields 101 a, 101 b. SQUID 103 is taken into a container 102 filled with liquid nitrogen 102a and arranged horizontally. The magnetization coils 106a, 106b are placed parallel mutually and have right angle to the SQUID 103.

The compensating coil 107 is placed in the lower part of the SQUID 103 and arranged parallel to the SQUID 103. Vertical component of the magnetic field generated by the magnetization coils 106 a, 106 b is canceled with the magnetic field formed by the compensating coil 107. Then the magnetic field inside the detection region includes only

horizontal flux substantially.

The transportation means 105 comprises an arm that moves to X-Y direction in horizontal and conveys a sample 104. Transportation means 105 can carry sample 104. The sample 104 is inserted into the magnetic shields 101 a, 101 b from side by the transportation means 105 and passes inside the coil 106 a, 106 b. Then the sample 104 is magnetized by the coil 106a, 106b. In next, the sample 104 arrives the detection region.

We assembled the apparatus mentioned above with elements below.

The SQUID 103 was made of patronized oxide superconducting thin film on a $SrTiO_3$ substrate. The magnetic shields 101 a, 101 b were made of Permalloy.

Sample 104 was supported by a glass plate having dimension of 20 mm *80 mm as a support 1. The glass plate is produced by Nalge Nunc International company (USA). The glass plate passed 1.5 mm lower part of the SQUID.

We prepared two kinds of antibody for preparation samples.

One is a A type antibody named "MACS" provided from Miltenyi Biotec company (Germany). The MACS is a particle of gamma-Fe_2O_3 14a coated by a polymer 14b and antibody 14 sticks to the polymer 14b as shown in figure 3 (a). Average particle diameter of the A type antibody is 50nm and weight of A type antibody is approximately $4*10^{-16}$ g.

Another one is a B type antibody named "dynabeads" provided by Dynal company (Norway). Plural magnetic material ultrafine particle 14a is contained in a polymer graining 14c as shown in figure 3(b) and an antibody 14 sticks to polymer 14c. Average particle diameter of B type antibody is 4.5 μm and weight of B type antibody is approximately

$14.3*10^{-12}$ g.

Example 1

Sample mentioned above was inspected with apparatus shown by figure 2. We used a decentralized liquid of A type antibody (rat / anti mouse Ig G1). In stock solution, concentration was indicated 0.2 mg/ml and Average particle diameter was 50nm, 5.2 g/cm^3. According to the inference, weight of magnetic material particle is $3.4*10^{-16}$ g and the particle is contained during stock solution at $5.8*10^{11}$ / ml. Then we diluted the stock solution with PBS into 1/10 and put it on the glass plate as an analyte. The sample on the glass plate occupied a region with 2 mm diameter and its amount was 2 μ liter. Accordingly, this sample contains $1.2*10^8$ magnetic particles and general mass of the magnetic particles is 40ng.

The acidity of magnetic field for magnetization was $8*10^{-4}$ T and the drift speed of analyte was 8 mm per second. Output signal of the SQUID 103 was recorded through a band-pass filter having range from 0.1 Hz to 5 Hz. Recorded output signal is shown in figure 4.

As shown in the figure 4, extremely clear variation of the magnetic field was recorded. Sensitivity of SQUID depends on the distance between a SQUID and an analyte. Therefore, the sensitivity of the apparatus can be regulated by the distance.

Example 2

A relation between the concentration and the detection resultant of the sample is shown in figure 5.

Circles plotted in the figure 5 show determination resultant of the sample that was labeled with the A type antibody and diluted with PBS in

various concentrations. Rectangles plotted in the figure 5 show determination resultant of the sample that was labeled with the B type antibody and diluted with PBS in various concentrations. The sample was rat / anti mouse Ig G1 and diluted with PBS. As shown in the figure 5, high correlation between the detected magnetic signals and the quantity of the labeled antibody can be seen for the both cases

Example 3

Another Sample was prepared. As shown in figure 6, in this sample, antigen 11 is fixed by a first antibody 12 to the support 1. Then, a second antibody 13 sticks selectively to the antigen 11. Furthermore, a third antibody 14 labeled with magnetic material 14a sticks to the second antibody 13. The SQUID detects the magnetic material 14a.

The sample put on the region having a diameter of 8 mm on the support. At first, we fixed a "mouse / anti humans interferon ß monoclonal antibody (YMASA company, JAPAN) as the first antibody 12 in region. Next, we let a humans-interferon ß as the antigen 11 react to the region at 37 degrees Centigrade for 3 hours. Then we prepared a rabbit-anti human interferon ß / polyclonal antibody (Bio-Rad company, U.S.A.) as second antibody 13 and goat / anti rabbit Ig G as the third antibody 14. The Goat / anti rabbit Ig G has been labeled with a magnetic material ultrafine particle and was reacted to the region at 37 degrees Centigrade for 1 hours.

Determination effect by measuring the sample above is shown in figure 7. The determination resultant is plotted with circles. At the same time, rectangles are plotted in the figure 7. These rectangles means resultant surveyed by an optical method according to a prior art, ELISA system type II by Biotrak company. In this method, at first, an antibody is reacted to an antigen and next, a stroma is added them. Then the

coloring antibody can be detected. For reference, the same goat / anti rabbit Ig G was used as an antibody.

As shown in the figure 7, this optical method shows good correlation with specified field where the concentration is more than 1 unit / ml. However, the correlation becomes worsen with lower field than the specified field. On the contrary, a good correlation is maintained by the method of the present invention. Then we understood the method of the present invention is clearly superior to the prior art. As explained, the method of the present invention can realize high sensitivity and high accuracy. Furthermore, a magnetic material can be smaller than 600pg, therefore, the sensitivity of the present invention should be improved easily.

Claims;

1. A method for immunoassay with magnetized label and SQUID, which comprising following processes;

(1) an analyte is labeled with a magnetic label to detect antigen-antibody reaction,

(2) the magnetic material label is magnetized by a magnetic field,

(3) the magnetized magnetic material label detected by a SQUID which detect a magnetic field having right angle to the magnetic field.

2. A method mentioned in claim 1, said magnetic field for magnetization is a static magnetic field.

3. A method mentioned in claim 1, said SQUID detects variation of the magnetic field occurred by moving the analyte labeled by magnetized magnetic material .

4. A method mentioned in claim 1, the analyte moves parallel to the magnetic field for magnetization.

5. An apparatus for immunoassay with magnetized label and SQUID, which comprising; a magnetic field generation means which generate a magnetic field to magnetize an analyte labeled by antigen-antibody reaction with magnetic material label, a SQUID which detects a magnetic field having right angle to the magnetic field generated by the magnetic generation means.

6. An apparatus mentioned in claim 5, the magnetic field generated by the generation means is a static magnetic field.

7. An apparatus mentioned in claim 5, which comprises a means conveying the analyte to pass trough the detection field of the SQUID.

8. An apparatus mentioned in claim 7, the analyte moves parallel to the first magnetic field.

9. An apparatus mentioned in claim 5, the magnetic field generation means generates second magnetic field which cancels component of the first magnetic field that is parallel to detecting direction of SQUID.

10. An apparatus mentioned in claim 5, the analyte is treated by a support which is sufficiently wide to the detection field of the SQUID.

11. An apparatus mentioned in claim 5, the SQUID is formed of an oxide superconducting thin film.

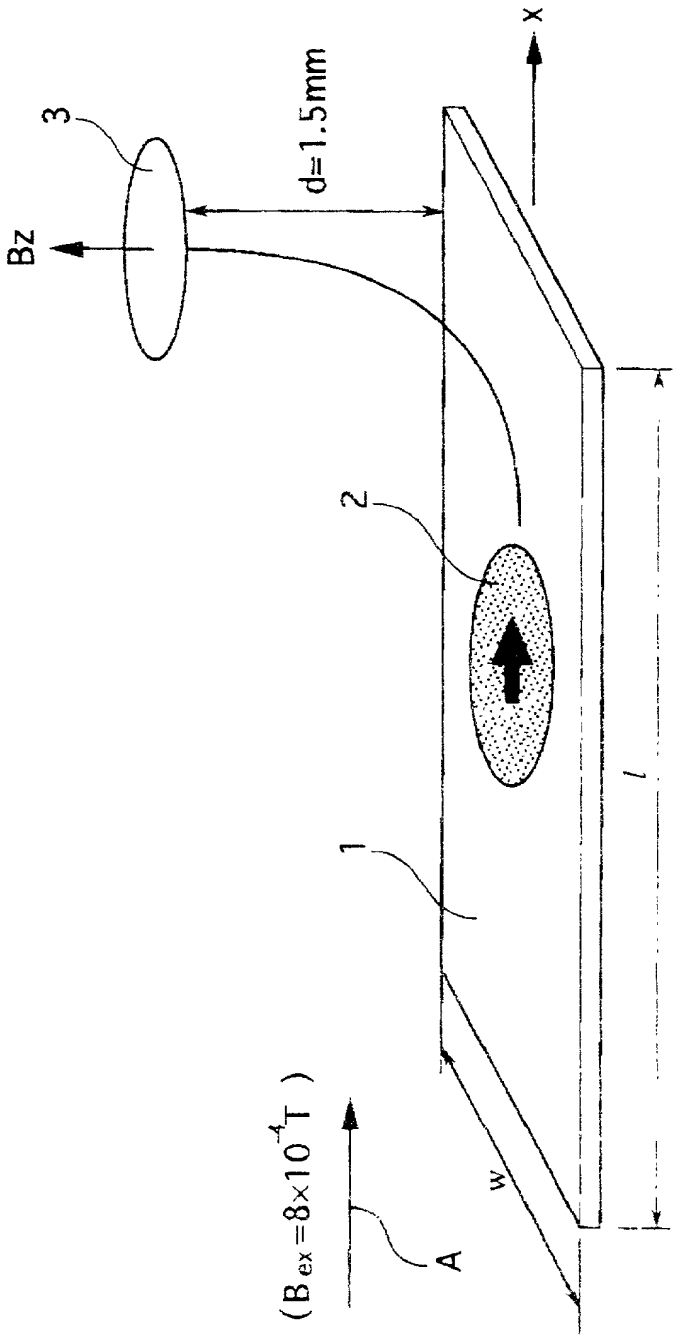
Abstract

The present invention relates to an immunoassay and an apparatus for the same.

A method of the present invention comprises following processes; (1) an analyte is labeled with a magnetic label to detect antigen-antibody reaction, (2) the magnetic material label is magnetized by a magnetic field, (3) the magnetized magnetic material label detected by a SQUID which detect a magnetic field having right angle to the magnetic field.

At the same time, the present invention contains an apparatus to execute the method provided by the present invention. The apparatus comprises a magnetic field generation means that generates a magnetic field to magnetize the labels. The apparatus comprises a SQUID that measures magnetic field.

Figure 1



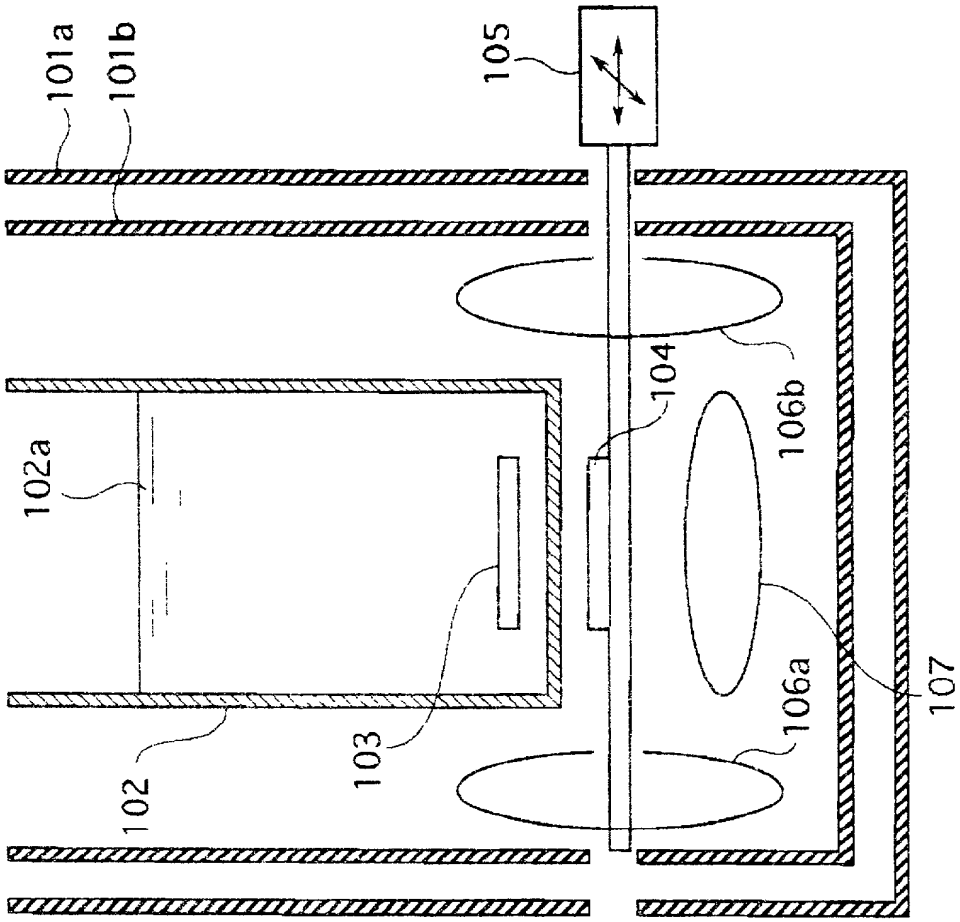
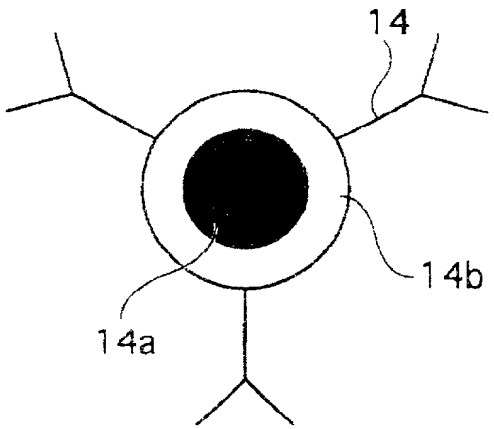


Figure 2

Figure 3

(a)



(b)

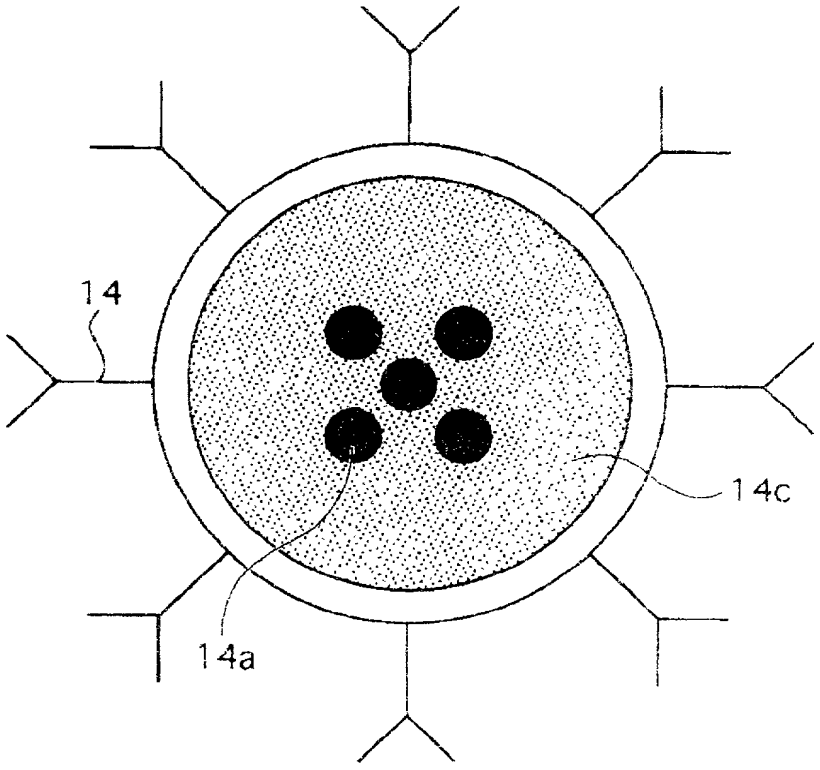


Figure 4

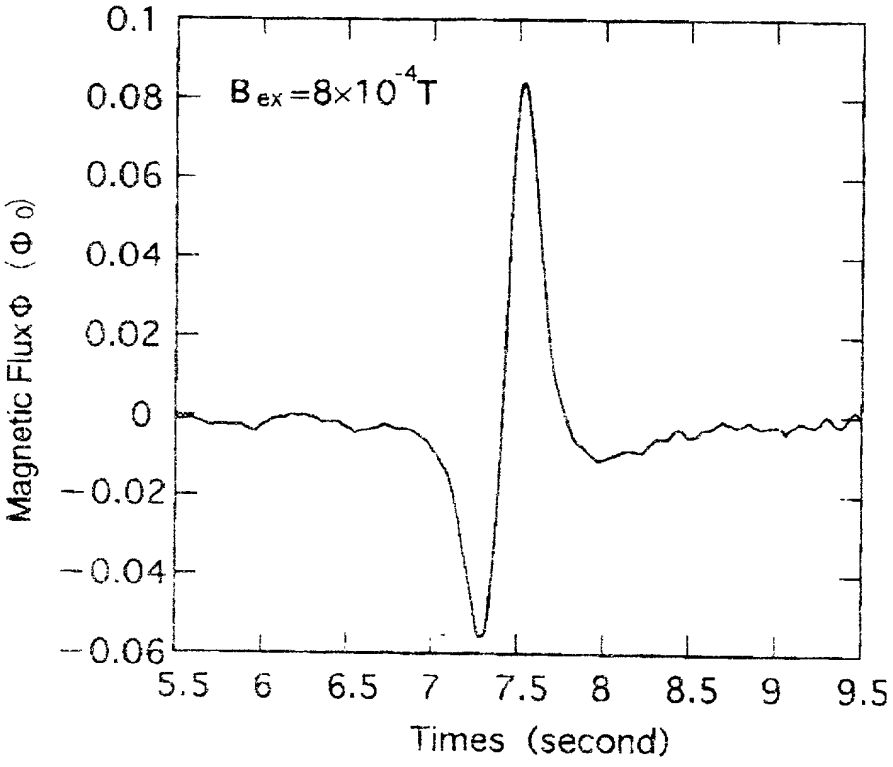
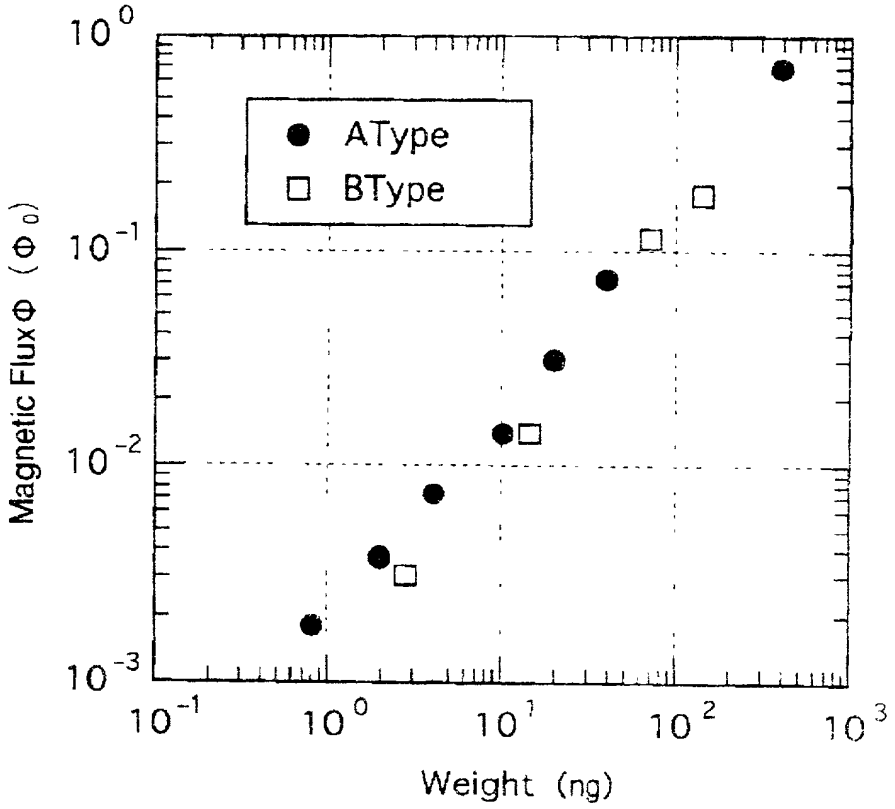

0.1
0.08
0.06
0.04
0.02
0
−0.02
−0.04
−0.06
$B_{ex}=8\times10^{-4}T$
Magnetic Flux Φ (Φ 0)
5.5
6
6.5
7
7.5
8
8.5
9
9.5
Times (second)

Figure 5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BType
Magnetic Flux Φ (Φ0)
Weight (ng)
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10^-2
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10^-1
10^0
10^1
10^2
10^3

Figure 6

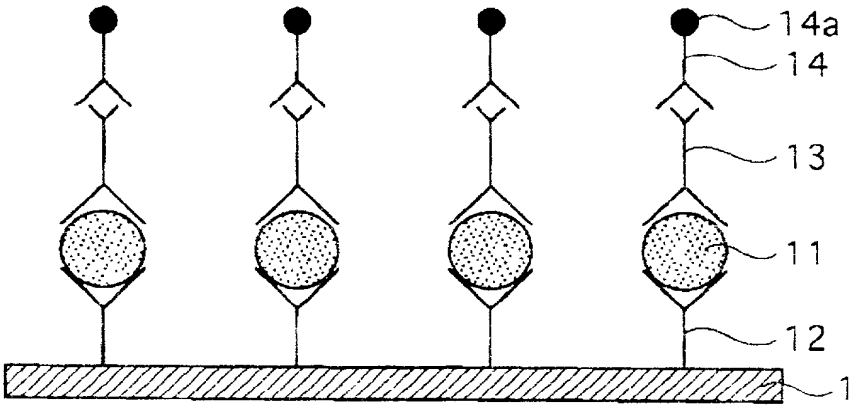
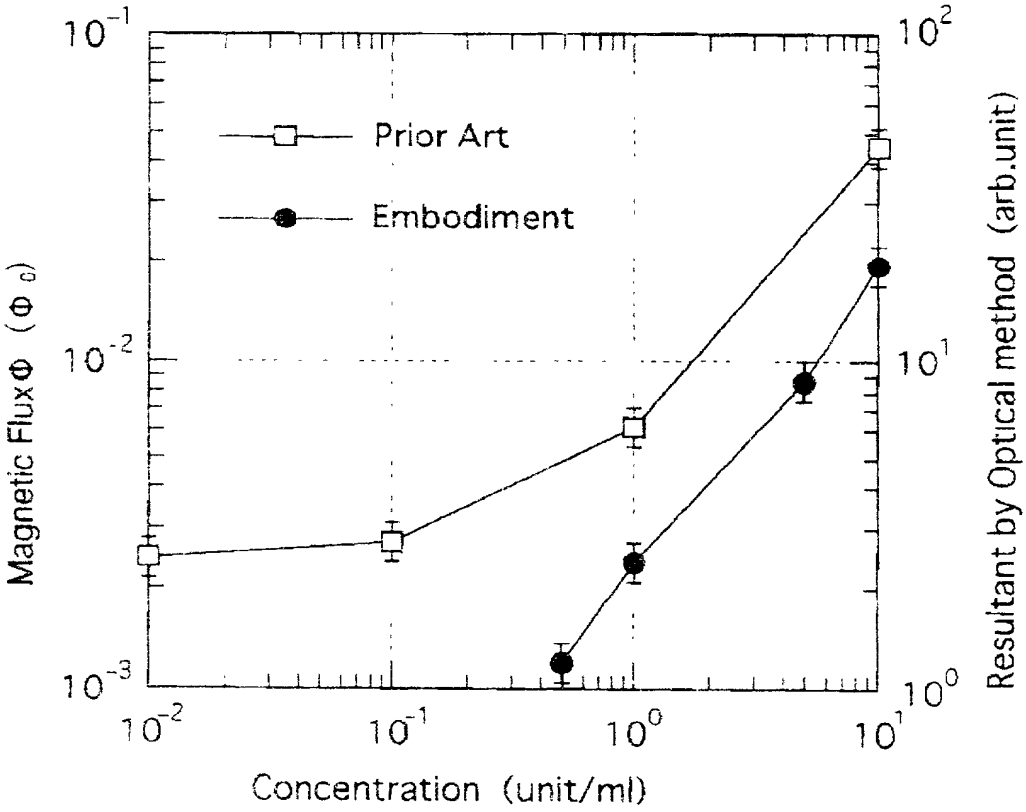


Figure 7



Docket No. _____

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

A METHOD FOR AN IMMUNOASSAY WITH A MAGNETIC LABEL AND AN APPARATUS FOR THE SAME

the specification of which is attached hereto unless the following box is checked:

☐ was filed on _____ as United States Application Number or PCT International Application Number _____ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
11-206248	JAPAN	21 July 1999	yes

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

APPLICATION NO	FILING DATE

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; William F. Ellis, Reg. No. 26,874; John J. Feldhaus, Reg. No. 28,822, Patricia D. Granados, Reg. No. 33,683, John P. Isaacson, Reg. No. 33,715, Donald D. Jeffery, Reg. No. 19,980; Eugene M. Lee, Reg. No. 32,039, Richard Linn, Reg. No. 25,144; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Sybil Melloy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298, Bernhard D. Saxe, Reg. No. 28,665; Charles F. Schill, Reg. No. 27,590, Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115, Harold C. Wegner, Reg. No. 25,258.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Residence Address	**Country of Citizenship**	
Post Office Address		

Full Name of Third Inventor	Signature of Third Inventor	Date
Residence Address	**Country of Citizenship**	
Post Office Address		

Full Name of Fourth Inventor	Signature of Fourth Inventor	Date
Residence Address	**Country of Citizenship**	
Post Office Address		

Full Name of Fifth Inventor	Signature of Fifth Inventor	Date
Residence Address	**Country of Citizenship**	
Post Office Address		